

Marked-up version for the last paragraph on page 12:

“As used herein, the term “sequence-specific DNA binding protein” refers to a protein that recognizes and binds a specific DNA sequence. The sequence bound by a sequence-specific DNA binding protein may be an invariant arrangement of contiguous nucleotide residues (e.g., GGATCC, SEQ ID No. 1) or it may be a conserved sequence motif in which individual residues may vary and still allow recognition and binding by the sequence-specific DNA binding protein (e.g., GGPuPyCC, SEQ ID No. 2 wherein Pu and Py are purine and pyrimidine, respectively). Binding of the protein to its specific sequence may be assessed via any conventional protein:nucleic acid binding methods, including but not limited to electrophoretic gel analysis of a given protein:nucleic acid construct.

Marked-up version for the last paragraph on page 18:

As used herein a "conditionally active transactivation domain of CHOP" encompasses amino acids 1-101 of the transcription factor CHOP. Specifically, the conditionally active transactivation domain of CHOP comprises the amino acid residues:

NH₃—

MAAESLPFTLETVSSWELEAWYEDLQEVLSSEIGGTYISSPGNEEEESKTFTTLD

PASLAWLTEEPGPTEVTRTSQSPRSPDSSQSSMAQEEEEEEQG-COOH (SEQ ID

No. 3)

and analagous sequences of transcription factor CHOP (for example, sequences that contain amino acid additions, insertions, deletions, substitutions) or other variations of CHOP that

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To facilitate the integration and selection for stable reporter gene integration, a hygromycin resistance expression cassette, excised from p3'SS (a vector for LacSwitchTM expression systems (Stratagene, GenBank Accession No. U42371), was inserted into the NdeI site of the pFR-Luc (Genbank Accession No. AF058756) luciferase reporter vector, to generate pFR-Luc-Hyg. pFR-Luc (and therefore pFR-Luc-Hyg) carries five copies of the GAL4 DNA-binding domain recognition sequence 5'-CGGAGTACTGTCCTCCG-3' (SEQ ID No. 4) upstream of a basic TATA element and the coding region for firefly luciferase (see Figure 4).

4.1.1. pFR-Luc Plasmid



Fig. 4

Sequence of GAL4 Binding Element in the pFR-Luc Plasmid

GT CCGAGTACTGTCCTCCG AG CCGAGTACTGTCCTCCG
 AG CCGAGTACTGTCCTCCG AG CCGAGTACTGTCCTCCG
 AG CCGAGTACTGTCCTCCG AG CCGAGACTCTAGAGGG
 TATATATGGATCCCCGGT AC CGAGCTCGAATTC-- (SEQ ID No. 5)
 --CAGCTTGGCATTCCGGTACTGTTGGTAAATG--Luciferase (SEQ ID No. 6)

4.1.2. Fusion Transactivator Plasmids

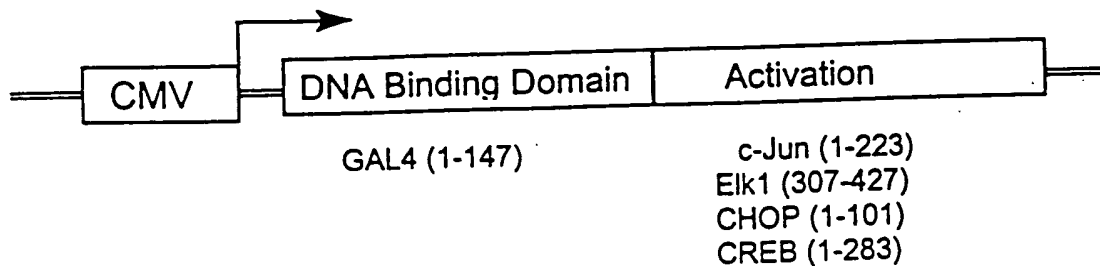


Figure 5

4.1.3. Control Plasmids

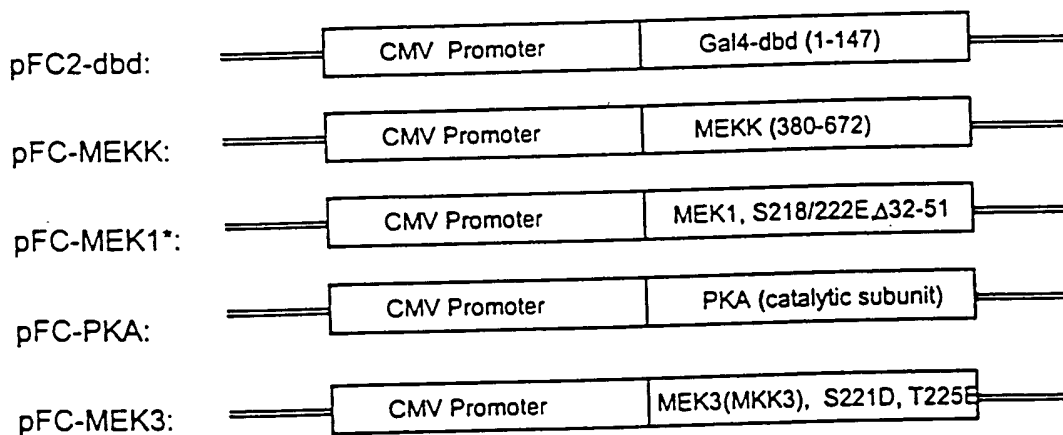


Figure 7

will tailor
to each application

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4.1.4. pFA-CMV Plasmid

pFA-CMV Plasmid

BamHI SrfI SmaI EcoRI XbaI HindIII PstI SacI KpnI BglII
 GTA TCG CCG GGA TCC GCC CGG GCT GGA ATT CTA GAA GCT TCT GCA GAG CTC GGT ACC AGA TCT TGA ATA AGT AG (SEQ ID No. 7)
 V S P G S G R A G I L E A S A E L G T R S * * * (SEQ ID No. 8)

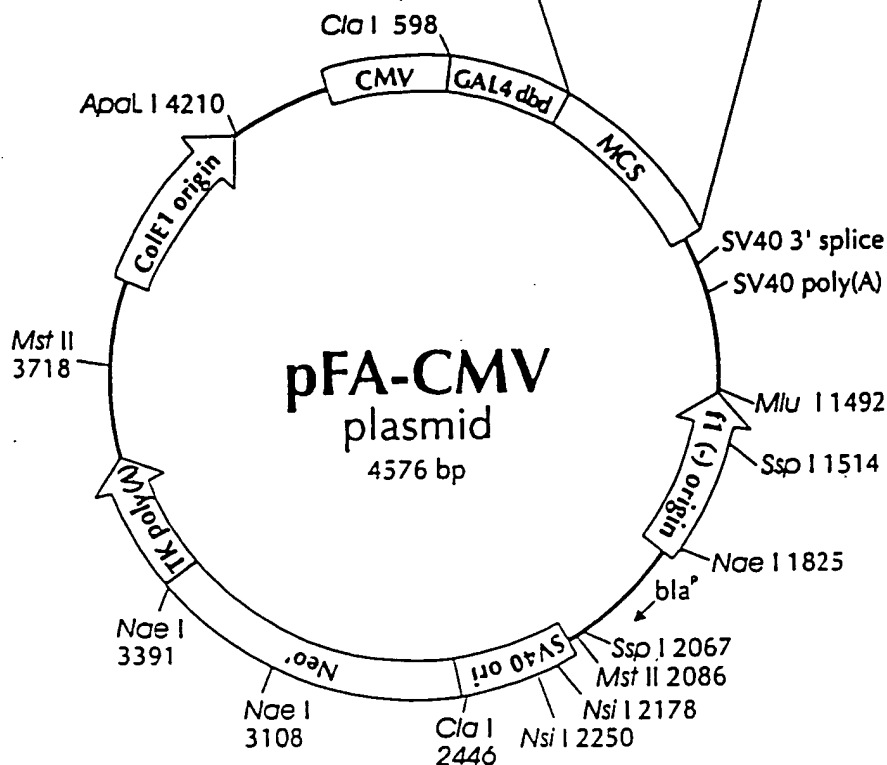


Figure 6

4.2. Preparation of medium and reagents

Luciferase Assay Reagent (1x)

40.0 mM tricine (pH 7.8)
 0.5 mM ATP
 10 mM MgSO₄
 0.5 mM EDTA
 10.0 mM DTT
 0.5 mM coenzyme A
 0.5 mM luciferin

Cell Lysis Buffer (5x)

40 mM tricine (pH 7.8)
 50 mM NaCl
 2 mM EDTA
 1 mM MgSO₄
 5 mM DTT
 1% Triton® X-100

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